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Transthyretin amyloidosis with pulmonary involvement in a patient with monoclonal gammapathy

Zmiany płucne w przebiegu amyloidozy z transtyretyny u pacjentki z gammapatią monoklonalną

The authors declare no financial disclosure

Abstract

Pulmonary involvement in the course of systemic senile amyloidosis caused by non-mutated transthyretin is rarely described. We report on concomitant monoclonal gammapathy of undermined significance (MGUS) and amyloidosis with non-mutated transthyretin with diffuse lesions in lung parenchyma.

A female patient, 67 years old, was admitted with dyspnoea, malaise, weight loss, and disseminated radiological lesions in the lungs. On lung HRCT, signs of pulmonary hypertension, alveolar and interstitial involvement, with thickening of septal lines were found. Echocardiography revealed severe pulmonary hypertension, and electromyography revealed sensoromotoric polyneuropathy with axon and myelin damage.

Pathological assessment of lung specimens revealed nodular deposits of amyloid in the bronchial walls and lung parenchyma Congo red staining was positive. Specimens of colon mucosa confirmed amyloidosis. Stainings for AA, AL and beta2-microglobulin were negative but were positive for transthyretin. Bone marrow trepanobiopsy indicated monoclonal gammapathy of MGUS type; Congo red staining was positive.

Transthyretin amyloidosis with vascular involvement, particularly of arteriovenous anastomoses, including pulmonary vessels and an insignificant amount of AL protein (perhaps secondary imbibition with AL protein from serum) was diagnosed in amyloid deposits. No mutations of the transthyretin gene (exon 1,2,3,4) were found.

The patient was treated with methylprednisolone, melphalan and then with cyclophosphamide. Radiological examinations performed 1 and 2 month/s after initiation of therapy showed progression of pulmonary lesions. The patient died one month later; an autopsy was not performed.

Key words: amyloidosis, MGUS, transthyretin, pulmonary involvement, respiratory failure

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Streszczenie

Zmiany płucne w przebiegu starczej amyloidozy z niezmutowanej transtyretyny są rzadko opisywane. Opisywany przypadek dotyczy współistnienia gammapatii monoklonalnej typu MGUS oraz amyloidozy z niezmutowanej transtyretyny z rozległym zajęciem miąższu płucnego.

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Sześćdziesięciosiedmioletnia kobieta została przyjęta na ddział z objawami duszności, osłabienia, utraty masy ciała oraz zmianami rozsianymi w płucach. W badaniu HRCT obecne były obszary wypełnień pęcherzykowych, pogrubienie linii przegrodowych oraz cechy nadciśnienia płucnego. W ECHO serca stwierdzono cechy znacznego nadciśnienia płucnego, badaniem elektromiograficznym czuciowo-ruchową polineuropatię z uszkodzeniem aksonu i mieliny.
Wykazano złogi amyloidu w formie guzków w ścianach oskrzeli oraz zrębie płuca, barwienie na czerwień Congo było dodatnie, ujemne dla białek AA, AL, beta2-mikroglobuliny, dodatnie dla transtyretyny. Amyloidozę potwierdzono również ze śluzówki jelita (ujemne barwienie dla białek AA, AL., beta2-mikroglobuliny, dodatnie dla transtyretyny). Trepanobiopsja szpiku wykazała gammapatię monoklonalną typu MGUS z dodatnim barwieniem na czerwień Congo. U chorej postawiono rozpoznanie amyloidozy z transtyretyny z zajęciem naczyń, w szczególności połączeń tętniczo-żylnych, w tym także płucnych z niewielką ilością białka AL (najpewniej była to wtórna imbibicja z surowicy krwi). Nie stwierdzono mutacji w egzonie 1, 2, 3, 4 dla genu transtyretyny. Pacjentka była leczona metyloprednisolonem, melfalanem oraz cyklofosfamidem. Zdjęcie przeglądowe klatki piersiowej wykonane 1 i 2 miesiące po rozpoczęciu terapii wykazało progresję zmian. Pacjentka zmarła w szpitalu rejonowym – sekcji zwłok nie wykonano.

Słowa kluczowe: amyloidoza, MGUS, transtyretyna, manifestacje płucne, niewydolność oddechowa
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Introduction

Amyloidosis is characterised by localized or systemic occurrence of amyloid deposits. The lungs may be involved in localized or systemic occurrence of amyloid deposits [1]. Pulmonary manifestations in the course of systemic amyloidosis caused by non-mutated transthyretin, a transport protein for thyroxine and retinol in plasma, are rarely reported. Lung involvement is seen more frequently in light chains amyloidosis (AL) [2]. Typical pulmonary lesions occurring in amyloidosis include localized nodules and diffuse infiltrates of alveolar septa [1–3]. The authors present a case report of concomitant MGUS-type (monoclonal gammapathy of underdetermined significance) monoclonal gammapathy and amyloidosis caused by non-mutated transthyretin with diffuse involvement of lung parenchyma.

Case Report

A female patient (G.K.), 67 years old, was admitted to the Division of Respiratory Diseases, University Hospital, Krakow, Poland, with symptoms of resting dyspnoea, malaise and weight loss progressing during the preceding few months. The patient status showed undernutrition (BMI 19). Chest examination revealed diastolic heart murmur in the pulmonary valve area and bibasilar rales. Arterial blood pressure was 160/100 mm Hg. Arterial blood gases indicated hypoxemic respiratory failure with pH 7.43, PaO_2 51.3 mm Hg and $PaCO_2$ 34.1 mm Hg. Pulmonary function tests showed a restriction pattern: FVC 59.8%, FEV_1 57.6% of predicted value, FEV_1/FVC 0.80 and TLCOc/VA 59.8 mmoL/min/kPa/L.

Laboratory assessment confirmed the presence of monoclonal protein in serum at concentration 16.2 g/L (total protein was 70.0 g/L), monoclonal protein in urine — kappa (κ) chains 37.4 mg/L (normal level: <7.08 mg/L), lambda (λ) chains 62.8 mg/L (normal level <3.89 mg/L), increased TSH level in serum (6.58 IU/mL, normal level 0.35 – 4.94 IU/mL) and decreased prealbumin level (0.170 g/L, normal level 0.18–0.36 g/L).

Chest X-ray revealed diffuse reticulo-nodular lesions in the lungs (Fig. 1). High resolution computed tomography (HRCT) showed thickening of septal lines, and areas of alveolar opacities and symptoms of alveolar and interstitial oedema (Fig. 2). Contrast-enhanced computed tomography demonstrated enlarged para-aortic and subcarinal lymph nodes. The features of the pulmonary hypertension were found with echocardiography: systolic pulmonary pressure of 95 mm Hg, considerably widened right ventricle and right atrium, paradoxical movement and shift to

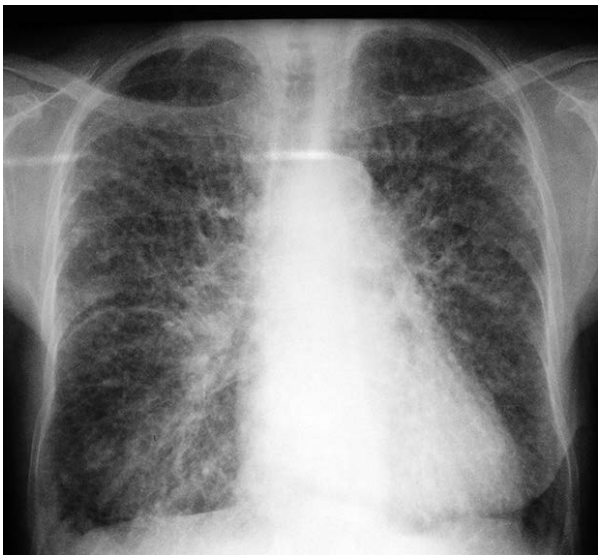

Figure 1. Chest X-ray showing diffuse reticulo-nodular lesions in the lungs

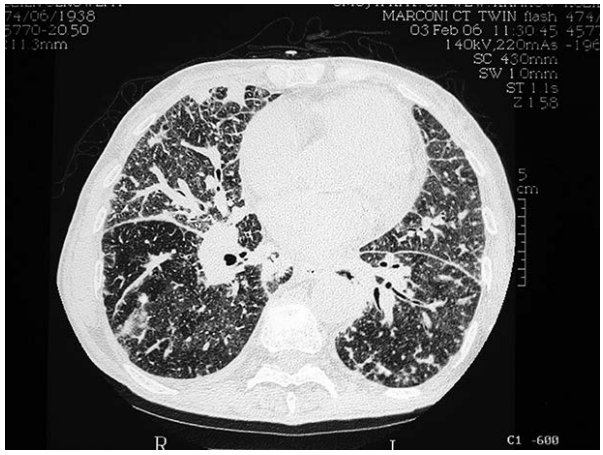

Figure 2. The HRCT scan with thickening of septal lines, areas of alveolar opacities and symptoms of alveolar and interstitial oedema

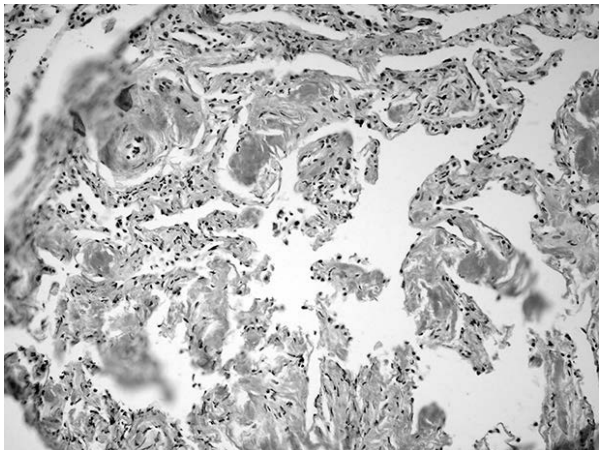

Figure 3. Positive Congo red staining in lung parenchyma in a specimen taken from the patient

left of interventricular septum and small left ventricle. Ejection fraction (EF) was 65%. Pulmonary angiography did not reveal embolic material in the pulmonary arteries, and this finding was concordant with the perfusion scintigraphy result.

As the patient complained of sensory paraesthesia of upper limbs, electromyography (EMG) was performed. It confirmed the presence of sensomotoric polyneuropathy with axon and myelin damage.

Bronchofiberoscopy with bronchial and transbronchial lung biopsy was performed.

Histopathological assessment of the biopsy samples revealed bronchial and lung amyloidosis with amyloid deposits in the form of nodules in the bronchial walls, lung parenchyma and arteriovenous anastomoses. Congo red staining (Fig. 3) as well as staining for transthyretin were positive (Fig. 4), but stainings for amyloid of serum protein A (AA) and for amyloid of light immunoglobulin chains (AL) were negative. A biopsy of rectal mucosa was also carried out and a similar staining pattern was obtained.

Following haematological consultation, the patient underwent trepanobiopsy of bone marrow, and MGUS-type monoclonal gammapathy was diagnosed. Congo red staining of the bone marrow biopsy specimen was also positive.

Thus the histopathological assessment of biopsies (lung, bronchi, colon mucosa, bone marrow) suggested transthyretin amyloidosis with vascular involvement with arterio-venous anastomoses, particularly in the lungs. A small amount of AL protein was also found in the amyloid deposits, but it was difficult to decide if this finding supported diagnosis of a complex type of amyloidosis or it was a result of a secondary infiltration with AL protein present in the serum.

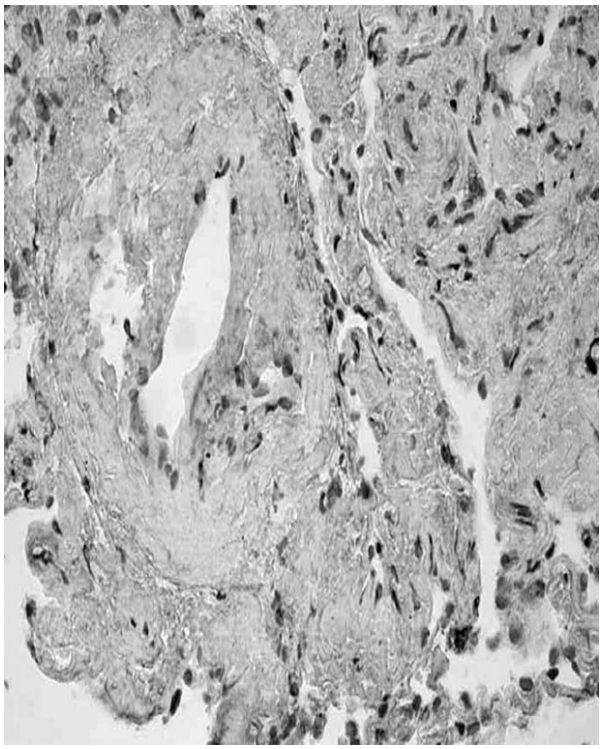

Figure 4. A specimen of lung parenchyma with positive staining for transthyretin

Genetic analysis was performed in order to determine the presence of mutations of the transthyretin gene (TTR, OMIM176300). The assessment of exons 2, 3 and 4 was performed in the Immunology Department, Hospital Clinic in Barcelona, Spain. After amplification with the PCR method, the Sanger method for direct sequencing of 2, 3 and 4 exons of the TTR gene was applied. The same approach was used for the analysis of exon 1 of the TTR gene in our

Laboratory of Molecular Biology and Clinical Genetics, Department of Medicine, Jagiellonian University Medical College, Krakow, Poland. No nucleotide changes that could be responsible for hereditary transthyretin amyloidosis were found in these four exons.

The patient was ordered chemotherapy with a regimen including methylprednisolone, melphalan, and cyclophosphamide. After two months of therapy, the patient was admitted to the Division of Respiratory Diseases because of severe respiratory failure (PaO_2 31.8 mm Hg, $PaCO_2$ 40 mm Hg). The laboratory results revealed a decrease of serum level of monoclonal protein (14.3 g/L) as well as κ and λ chains levels in urine (25.4 and 49.5 mg/L, respectively). Chest X-ray and HRCT showed intensive progression of interstitial lesions in the lungs. A further increase of pulmonary artery pressure to 100 mm Hg was confirmed with echocardiography. There were no symptoms of pulmonary embolism in CT angiography. Bacterial cultures from bronchial washings were negative. The patient was treated with clarithromycin, calcium channel blocker, beta-blocker, angiotensin-converting enzyme inhibitor, diuretics and oxygen therapy. Improvement of the patient's status and arterial blood gases was achieved, but there was no change in radiological image. The patient was discharged from the Division of Respiratory Diseases for further ambulatory treatment and long-term oxygen therapy at home. One month later the patient was admitted to the regional hospital with symptoms of severe respiratory failure and died despite symptomatic treatment. An autopsy was not performed at the family's request.

Discussion

Amyloidosis is characterised by the presence of extracellular deposits of insoluble fibrillar protein in one or many organs. Amyloid deposits are identified under polarized light as they yield green birefringence following Congo red staining. Furthermore, under electron microscopy they are seen as straight, non-branching fibrillae of length 8-10 nm. Fibrillae are composed of 5–25 kD subunits of various circulating plasma proteins. At present, about 25 amyloid protein precursors have been identified. The classification of amyloidosis is based on identification of these precursors [4, 5].

The two most common types of systemic amyloidosis are light chain amyloidosis (AL) with a prevalence of 1 case per 100 000 and secondary (reactive) amyloidosis (AA) caused by chronic inflammatory diseases (e.g. rheumatoid polyarthritis, chronic infections) [4–6].

Familial amyloidosis is a group of hereditary disorders with an autosomal dominant mode of inheritance. Amyloid fibrillae are formed from mutated proteins, and the process usually starts in middle age (4th or 5th decade). The most common type of familial amyloidosis is caused by mutated transthyretin. Other types of hereditary amyloidosis result from mutation of apolipoprotein A-I, gelsoline, alpha chains of fibrinogen A and lysozyme [4, 7–10].

Senile systemic amyloidosis prevails in subjects above 75 years old and is associated with systemic deposition of non-mutated, transthyretin (mainly in the heart and blood vessels) [4, 9–11].

Clinical symptoms depend on the type of precursor protein, its tissue deposition and the amount of amyloid. In the two main types of amyloidosis, primary (AL) and secondary (AA), amyloid accumulates mainly in the kidneys, heart, liver and nervous system and leads to organ failure [4, 9].

The respiratory system is usually involved in AL amyloidosis. It results in the occurrence of tracheobronchial infiltrates, nodular lesions or alveolar filling deposits in lung parenchyma, pleural effusion and/or mediastinal lymph node involvement [3, 4, 9]. Multifocal submucosal infiltrates, nodules or tumour-like structures are typical for tracheobronchial disease. This type of amyloidosis often coexists with osteoplastic tracheobronchopathy. Clinical manifestations include dyspnoea, cough and haemoptysis. Pulmonary disease is frequently accompanied by amyloidosis of other organs. The radiological nodular or alveolar filling pattern may resemble other interstitial lung diseases or pulmonary oedema. Involvement of the lymphatic system is typically associated with amyloid deposits in mediastinal lymph nodes. It may accompany a B-cell lymphoma. Involved lymph nodes may contain calcifications [9]. Lungs are extremely rarely involved in AA, and hereditary types of [2] amyloidosis caused by mutated transthyretin (equivalent for prealbumin) affect mainly the heart. It may also lead to familial polyneuropathy or nephropathy [4, 5, 7–9, 11].

Pulmonary involvement may also occur in senile systemic amyloidosis caused by deposits of non-mutated transthyretin in heart, kidneys, and intestine. This type of amyloidosis is found mainly in elderly patients, usually above 75 years old [4, 9–11]. In the reported case of a 67-year-old female patient, we investigated clinical and radiological features of interstitial lung disease with increased serum level of monoclonal protein and increased urine levels of λ and κ chains. Histo-

pathological assessment of the samples obtained with bronchial and transbronchial lung biopsy yielded the diagnosis of amyloidosis caused by transthyretin deposits (positive Congo red staining, negative staining for AL and AA). The diagnosis was confirmed by histopathological assessment of colon mucosa biopsy. Trepanobiopsy of bone marrow yielded a diagnosis of MGUS; staining with Congo red was also positive.

MGUS is the form of monoclonal gammapathy associated with paraproteinemia. MGUS occurs in 1–2% of patients above 60 years old and in 4–5% of those above 80 years old. Annual risk of neoplastic transformation is 1%. It is characterised by the lack of lytic lesions in bones, no symptoms of chronic infections, IgG M-component < 30 g/L, and percentage of clonal plasma cells in bone marrow < 10% [12].

The criteria for diagnosis of multiple myeloma were not fulfilled in our patient. After haematological referral, chemotherapy was initiated in the patient because of diffuse organ involvement. Following two series of chemotherapy, the progression of pulmonary lesions was observed even though a decrease of serum monoclonal protein and urine levels of kappa and lambda chains was obtained. The patient died with symptoms of circulatory and respiratory failure. While discussing differential diagnosis, we assumed that MGUS would be an improbable cause of pulmonary lesions leading to respiratory failure and severe pulmonary hypertension in this patient. Histochemical tests were positive for transthyretin. Although a small amount of AL protein was found in amyloid deposits, it could be probably attributed to secondary imbibition of plasma AL protein. Chemotherapy did not bring any improvement in lung involvement; on the contrary, rapid progression was observed during the treatment. We believe that the patient, despite her relatively young age (67 years old), suffered from systemic senile amyloidosis with associated involvement of bronchial walls and lung parenchyma, as well as the presence of arterio-venous anastomoses, which were responsible for the severe pulmonary hypertension observed in advanced amyloidosis [13]. Amyloid deposits were also present in the gastrointestinal tract.

Pulmonary hypertension in patients with amyloidosis is most frequently caused by restrictive cardiomyopathy related to involvement of the left ventricle. In our patient the left ventricle was not compromised by amyloidosis. Thus, hypoxaemia resulting form amyloid interstitial infiltrations of interstitial lung tissue remains the most probable cause. However, pulmonary hypertension in amyloidosis may also occur without involvement of the left ventricle or lung interstitium [14].

It is also possible that the patient was affected by mixed type amyloidosis caused by light chains and non-mutated transthyretin despite the low amount of AL amyloid in pulmonary tissue. This could be related to a loss of amyloid epitopes leading to insufficient immunohistochemical reactions. Cases of amyloidosis associated with simultaneous occurrence of light chains and mutated transthyretin deposits were reported in literature [15]. Similar phenomenon could possibly occur in senile systemic amyloidosis. Unfortunately, an autopsy was not performed on the patient and this precluded ultimate diagnosis.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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